Ed Loth

UNITED STATES GOVERNMENT

memorandum

DATE:

July 15, 1988

REPLY TO

Chris Ingersoll, Section Leader Fish and Invertebrate Toxicology Section

SUBJECT:

Final report on the "Effluent Toxicity Testing of Agricultural Irrigation Drainwater at Stillwater National Wildlife Refuge"

TO:

Buckler, Burch, Cleveland, Coyle, Dwyer, Fabacher, Finger, LaPoint, Lemly, Little, Mauck, Mehrle, Nelson, Schoettger, Schwartz, Steadman

Attached is a copy of the final report for the acute toxicity studies conducted by our Section with samples collected from Stillwater. This report was sent to: (1) Wally Steucke, Regional Director, FWS, Region 1, Portland, OR (2) Clarence Johnson, Office of Research Support Region 8, Washington, DC, (3) Richard Stroud, Environmental Contaminants Coordinator, Region 1 - Regional Office, Portland, OR and (4) Ron Anglin, Refuge Manager, Stillwater NWR, Fallon, NV.

Thanks,

Chris

Chris

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WHOLE EFFLUENT TOXICITY OF AGRICULTURAL IRRIGATION DRAIN WATER
ENTERING STILLWATER NATIONAL WILDLIFE REFUGE, NV:
ACUTE TOXICITY STUDIES WITH FISH AND AQUATIC INVERTEBRATES

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July 14, 1988

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Ingersoll, C.G., F.J. Dwyer, M.K. Nelson, S.A. Burch, and D.R. Buckler.
Whole Effluent Toxicity of Agricultural Irrigation Drain Water entering
Stillwater National Wildlife Refuge, NV: Acute Toxicity Studies with
Fish and Aquatic Invertebrates.

SUMMARY

The objective of these studies was to investigate the potential acute toxicity of agricultural drain water entering Stillwater National Wildlife Refuge (SNWR) to freshwater fish and invertebrates. Drain water was collected by SNWR personnel from four sites (Pintail Bay, Lead Lake, Hunter Drain, and TU Drain) and shipped to the National Fisheries Contaminant Research Center (NFCR) for acute effluent toxicity testing. In addition, dilution test water was obtained from a reference site (Lahontan Lake) near SNWR. Both freshwater and saltwater cultured/acclimated fish and invertebrates were used to assess the acute toxicity of these effluents. The use of both freshwater and saltwater organisms allowed for the separation of toxic effects due to salinity from the toxic effects of additional contaminants associated with the agricultural irrigation water entering SNWR. Water collected from Pintail Bay was acutely toxic to both freshwater and saltwater cultured/acclimated fish and invertebrates; water from TJ Drain was toxic only to the freshwater cultured animals and saltwater cultured D. magna. The toxicity of Pintail Bay effluent was probably a function of both inorganic contamination and elevated salinity. The toxicity of TU Drain effluent was probably more directly related to salinity. The toxic mechanism occurring within complex chemical mixtures cannot always be identified on the basis of single-compound toxicity values because of possible interactions among the major identified and unidentified contaminants. Additional studies are needed using reconstituted mixtures of principal chemical components to identify key toxicants and their interactions with other mixture constituents.

INTRODUCTION

Contaminants in agricultural irrigation drain water pose a threat to fish and wildlife resources at several sites administered by the U.S. Department of the Interior (DOI). Recently, DOI formed a Task Group of Irrigation Water Quality which is charged with evaluating existing and potential drain water related contaminant problems on DOI lands throughout the western states. The results of initial field investigations indicate a need to further assess biological impacts at four locations: Stillwater in

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Nevada, Ouray in Utah, Salton Sea in California, and Kendrick in Wyoming (Lemly 1987).

The objective of the present studies was to determine the acute toxicity of four separate agricultural drain waters entering Stillwater National Wildlife Refuge. The biological significance of drain water contaminants in aquatic ecosystems are often unknown. Because most drain water contains several potentially toxic substances, it is difficult to characterize environmental hazard on a site-specific basis by examining individual contaminants. The potential for antagonistic and/or synergistic interactions of complex contaminant mixtures must be considered in the hazard assessment. In such cases, approaches are needed that evaluate whole effluent toxicity of the drain water. Both freshwater and saltwater organisms were tested in order to separate the toxic effects associated with elevated salinity from effects associated with additional contaminants. The species tested included: striped bass (Morone saxatilis), fathead minnows (Pimephales promelas), amphipods (Hyalella azteca), and cladocerans (Daphnia magna).

All of the studies described in this report were conducted using NFCR Protocols which are based on the Good Laboratory Practice outlined in the Federal Register (160.120; 40 CFR Ch.1; 7-1-85 edition; subpart G -"Protocol for and conduct of a study"). These studies are part of a series of studies being conducted to determine the toxicological significance of contaminants associated with agricultural drain water. A detailed description of the Protocols is outlined in NFCR Research Study Plan 890.02: "Toxicological Evaluation of Contaminants Identified through RCA Surveys as Potential

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Problems for Aquatic Resources." For a copy of the Research Study Plan or of the Protocols used to conduct these studies contact C. Ingersoll.

MATERIALS AND METHODS

Collection, shipment and preparation of water samples

Thirty liters of water was collected from each of four sites located in SNWR: (1) Lead Lake, (2) Pintail Bay, (3) Hunter drain, and (4) TJ drain on May 22, 1988. In addition, 200 L of dilution test water was obtained from a reference site (Lahontan Lake) located near SNWR. The water samples were placed in polyethylene carboys and chilled to about 4°C by SNWR personnel under the direction of Steve Thompson (Assistant SNWR Manager). These carboys were shipped by air freight in coolers to NFCR on May 23rd and were held at 4°C until the initiation of the toxicity tests on May 24th.

On the day the exposures were initiated (Day 0), the water from each site was aerated to achieve thorough mixing and saturation of dissolved oxygen. A 50% serial dilution of each 100% test site water was made with the dilution (Iahontan Iake) water. Four dilutions were tested: 100%, 50%, 25%, and 12.5% site water, plus the reference site (dilution water) and a laboratory culture water control, for a total of six treatments. Dilution test waters were mixed in five-gallon plastic containers before distribution into the toxicity testing chambers.

Toxicity testing

Two fish species were tested in 96-hour acute effluent toxicity tests: striped bass (Morone saxatilis; ~0.2 g fish, 6 weeks post-hatch) and fathead minnows (Pimephales promelas; <48 h post-hatch). In addition, two species of invertebrates: the amphipod Hyalella azteca (<3 mm, second instar) and

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the cladoceran Daphnia magna (<24 h old) were tested in 72-hour and 48-hour acute toxicity tests, respectively (USEPA 1985a). These animals are routinely cultured at NFCR using appropriate Standard Operating Procedures (SOP's). The fathead minnows, \underline{H} . \underline{azteca} , and \underline{D} . \underline{magna} were cultured in NFCR fresh water (hardness 272 mg/L as $Caccoloring_3$) before the initiation of the exposures. In addition, separate groups of H. azteca and D. magna were: cultured in 12 parts per thousand (ppth) and 5 ppth salt water, respectively, and the striped bass were acclimated to 12 ppth salt water for 3 days before the initiation of the exposures. The salt water was reconstituted using appropriate amounts of Instant Ocean^R salts added to a mixture of 25% well water:75% reverse osmosis/deionized water. In total, six different toxicity tests were conducted with water collected from the four drain water sites: (1) striped bass acclimated to 12 ppth salt water, (2) fathead minnows cultured in fresh water, (3) H. azteca cultured in fresh water, (4) H. azteca cultured in 12 ppth salt water, (5) D. magna cultured in fresh water, and (6) D. magna cultured in 5 ppth salt water.

Each toxicity test was initiated with ten animals per test concentration. Fathead minnow tests were conducted in 1-L glass beakers using 750 mL of test solution. Striped bass tests were conducted in 19.6-L glass jars using 5 L of test solution. D. magna and H. azteca toxicity tests were conducted in separate 250-mL glass beakers using 200 mL of test solution. Mortality was recorded every 24 hours. For invertebrates, death was defined as a cessation of all visible signs of mobility during a 5-second observation after prodding the test animal with a blunt probe. Animals were not fed during the exposures. All tests were conducted at

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20°C. Ambient lighting was used during the fish tests. The photoperiod was 16:8 h light:darkness for the invertebrate tests.

Chemical analysis

Dissolved oxygen (D.O.), salinity, conductivity, pH, total water hardness (as CaO₃), alkalinity (as CaO₃), chloride, sulfate, and osmolality were measured on Day 0 for the four effluents (100% site water), the reference site and the laboratory culture water controls using standard methods (e.g. APHA 1985), NFCR Good Laboratory Practice (GLP), and NFCR SOP's (Table 1). Water quality (D.O., pH, salinity, conductivity) was monitored for the four test effluents at 100% and 25%, the reference site, and the laboratory culture water controls at the beginning and end of the tests.

Inductively coupled argon plasma emission spectrophotometry (ICAPES) (Jarrel-Ash Model 975) was used to obtain multiple element analysis of each undiluted water sample (Table 2). Water samples were withdrawn for ICAPES chemistry residue analysis from the four effluents (100% site water), the reference site, and the three laboratory culture water controls. Polyethylene bottles (I-Chem) were used to hold 250 mL of filtered (0.4 µm Nuclepore^R polycarbonate filter) water. All samples were preserved with concentrated HCl to pH <2 before ICAPES analysis. Of the samples analyzed, 10% were blanks and 20% were blind replicates and spiked samples. No values for blanks exceeded the detection limits for any element (Table 2). All quality assurance spiked samples were within 20% of certified concentrations for the reference materials. Analyses were performed by the Environmental Trace Substances Research Center, University of Missouri, under contract specified by the U.S. Fish and Wildlife Service.

Statistical analysis

Where mortality was greater than 50% by the end of the exposure period, the IC50 was estimated using either probit, binomial, or moving average methods as appropriate (Stephan 1977). Where an IC50 could not be calculated (e.g. no partial mortality) a range encompassing the probable IC50 was reported.

RESULTS

Water chemistry

Measured pH, alkalinity (as CaOO3), hardness (as CaOO3), conductivity, salinity, D.O., sulfate, chloride, and osmolality for each test site, the reference site (Iahontan), and the laboratory culture water controls are listed in Table 1. During the studies D.O. remained at greater than 50% of saturation; salinity, pH, and temperature remained relatively constant during the exposures. Results from the ICAPES analysis for each test site, the reference site and the laboratory culture water controls are listed in Table 2. Vanadium, chromium, and lead concentrations were less than 0.05, 0.1, and 0.04 mg/L, respectively, for all water samples; selenium was <0.5 mg/L for TJ Drain, Pintail Bay and 12 ppth control salt water, and <0.05 mg/L for the remaining water samples.

Toxicity testing

Striped bass: Only water from Pintail Bay was acutely toxic to larval striped bass (Table 3). The 96-hour LC50 ranged between 50 and 100% Pintail Bay effluent for saltwater acclimated striped bass, which corresponds to 12 to 23 ppth salinity. Up to 30% mortality was observed in

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several of the dilution series for the other three sites, however, control mortality was about 10-20% in these exposures.

Fathead minnows: Water from both Pintail Bay and the TU Drain was toxic to freshwater cultured larval fathead minnows (Table 4). The 96-hour IC50 for fathead minnows was 26% effluent for Pintail Bay (~6 ppth salinity) and 28% effluent for TU Drain (~5 ppth salinity). In addition, survival of fathead minnows in Iahontan Iake water was only 40% for the 96-hour exposure. The calculated IC50 values for fathead minnow larvae may need to be interpreted with caution because of this mortality in Iahontan Iake water, however, 100% effluent from both Pintail Bay and TU Drain was clearly toxic to fathead minnow larvae.

Amphipods: The response of <u>H</u>. <u>azteca</u> was quite similar to striped bass. Only water from Pintail Bay was acutely toxic to juvenile amphipods (Table 5). The 72-hour IC50 for Pintail Bay was 31% effluent (77 ppth salinity) and 35% effluent (8 ppth salinity) for saltwater and freshwater cultured <u>H</u>. <u>azteca</u>, respectively.

Daphnids: The response of <u>D. magna</u> was quite similar to the fathead minnows. Water from both Pintail Bay and TJ Drain was toxic to both saltwater and freshwater cultured daphnids (Table 6). The 48-hour IC50 for Pintail Bay was 35% effluent (~8 ppth salinity) for both saltwater and freshwater cultured <u>D. magna</u>. The 48-hour IC50 for TJ Drain was 38% effluent (~7 ppth salinity) and 35% effluent (~7 ppth salinity), respectively for <u>D. magna</u> cultured in salt water and fresh water.

DISCUSSION

Water collected from Pintail Bay was acutely toxic to both freshwater and saltwater cultured/acclimated fish and invertebrates; water from TU Drain was toxic only to the freshwater cultured animals and saltwater cultured D. magna. The toxicity of Pintail Bay effluent was probably a function of both inorganic contamination and elevated salinity. The toxicity of TU Drain effluent was probably more directly related to salinity.

Survival of fathead minnows and freshwater or saltwater cultured daphnids was reduced with exposure to either Pintail Bay or TJ Drain effluent at dilutions corresponding to about 5-8 ppth salinity. However, survival for both of these species was not reduced with exposure to Hunter Drain effluent at a salinity of about 9 ppth. Survival of striped bass and freshwater or saltwater cultured amphipods was reduced with exposure to Pintail Bay effluent at about 12-23 ppth and 7-8 ppth salinity, respectively. Yet, survival of these two species was not reduced with exposure to TJ Drain effluent at 19 ppth salinity or Hunter Drain effluent; at 9 ppth salinity. Clearly the toxicity of the drain waters cannot be explained by salinity stress alone.

Striped bass and <u>H. azteca</u> are both tolerant of elevated salinity.

Otwell and Merriner (1985) report that striped bass survival and growth were not adversely affected when 1-2 month old fish were transferred directly from fresh water to 20 ppth salinity for 7 days. <u>H. azteca</u> inhabit freshwater to estuarine/brackish habitats up to 22.5 ppth salinity (Thomas 1976, Timms et al. 1986). In addition, we routinely culture and conduct tests with this amphipod at salinities up to 17 ppth with no indication of

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salinity stress. In contrast, fathead minnows and <u>D. magna</u> are relatively intolerant of elevated salinity. Meyer et al. (1985) and Adelman and Smith (1976) report acute lethality for either <u>D. magna</u> or fathead minnows at about 6-7 ppth salinity.

While several inorganic contaminants were elevated in the drain water effluent samples (Table 2), none of these elements were present at acutely lethal concentrations based on reported literature values for singlecompound toxicity tests. In the present study, concentrations of As (USEPA 1985b), B (Gersich 1984), Cd (Ingersoll and Winner 1982), Cr (USEPA 1985c), Cu (Ingersoll and Winner 1982), Pb (USEPA 1986), Li (McKee and Wolf 1963), Mo (McKee and Wolf 1963), Se (Ingersoll et al. 1988), Sr (McKee and Wolf 1963), V (Beusen and Neven 1987), Zn (USEPA 1986) (Table 2) and sulfate (Table 1) (McKee and Wolf 1963) were well below reported acutely lethal levels to fish or aquatic invertebrates. Mercury concentrations were not determined for any of the water samples in the present study, however, a previous study of selected sites from SNWR reported Hg concentrations were <0.9 ug/L (Steve Thompson SNWR personal communication) which is well below the reported acute toxicity of Hg to most aquatic organisms (USEPA 1986). The concentrations of organochlorine pesticides and nitrogenous compounds were not determined in the present study and were not reported in previous studies dealing with SNWR (Steve Thompson SNWR personal communication). Perhaps organic contaminants, nitrogenous compounds and additional undetermined inorganic contaminants may contribute to the acute effluent toxicity of Pintail Bay and the TU Drain. The on-site studies to be conducted at SNWR by NFCR later this summer will further elucidate the chemical nature and toxicity of the drain water.

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The toxic mechanism occurring within complex chemical mixtures cannot always be identified on the basis of single-compound toxicity values because of possible interactions among the major components (Meyer et al. 1985). Additional studies are needed using reconstituted mixtures of principal chemical components to identify key toxicants and their interactions with other mixture constituents. The on-site toxicity studies planned by NFCR will determine the potential toxicity of additional drain waters entering SNWR. Additional studies are needed to develop a complete hazard assessment of the sources, fate and potential chronic impacts of contaminants associated with drain water and sediment on freshwater fisheries and wildlife resources at SNWR. An integration of knowledge of effects with the distribution, bicavailability and persistence of contaminants associated with drain water entering SNWR is needed.

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Table 1. Measured water quality characteristics at the beginning of the exposures (Day 0) from the designated sites or controls.

Site/Control	Percent Site Water	рΉ	Alkalinity (mg/L ^a)	Hardness (mg/L ^a)	Conductivity (µmhos/cm)	Salinity (ppth)	D.O. (mg/L)	Sulfate (mg/L)	Chloride (mg/L)	Osmolality (mmol/Kg)
Lead Lake	12.5%				1,103	0	10.0	···········!		
	25.0%	8.59			1,366	0	10.2			
	50.0%				2,337	0	10.2			
	100.%	8.92	217	460	4,144	1 2	10.0 10.0	538	988	60
TU Drain	12.5%				E 040	•			200	
	25.0%	8.40			5,843	3	10.0			
	50.0%	00.00			8,691	5	10.0			
	100.8	8.23	369	3780	15,674	11	9.8			
		0.20	305	3760	28,738	19	9.2	3,019	10,400	503
Hunter Drain	12.5%				2,670	1	0 5			
	25.0%	8.30			3,552	1	9.5			
	50.0%		•		6 , 867	2	9.7			
	100.%	8.05	183	740	12,655	4	9.5			
			200	740	12,000	9	9.4	835	4,120	194
Pintail Bay	12.5%				6,630	4	٥			
	25.0%	9.46			8,796	4	9.5	÷		
	50.0%				16,458	6	9.5			
	100.%	9.54	974	830	30,306	12	9.5			
			- , .	050	30,306	23	9.4	2,659	11,180	542
Lahontan Dam	100.%	8.55	93	91	242	0	10.5	38	0.0	
~					5.5	J	10.5	38	20	<15
Control Fresh	100.8	8.43	272	297	470	0	8.8	46	31	<15
Control Salt				,					31	\1 3
				<u>:</u> ·			/			
5 ppth	100.%	8.31	140	1250	9,000	5	8.4	446	3,901	171
12 ppth	100.%	8.31	140	2550	20,846	12	8.1	998	6,920	439

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Table 2. Concentration (mg/L) of arsenic (As), boron (B), calcium (Ca), cadmium (Cd), copper (Cu), lithium (Li), magnesium (Mg), molybdenum (Mo), potassium (K), sodium (Na), strontium (Sr), and zinc (Zn) in water samples from the designated site or control.

Site/Control	As	В	Ca	Cd	Cu	Li	Mg	Мо	K.	Na	Sr	Zn
Lead Lake	0.08	3.85	51	0.004	0.015	0.246	66	0.083	22.0	724	1.34	0.11
TU Drain	<0.4	19.0	379	<0.03	0.02	0.90	511	0.70	69.0	4940	10.1	<0.03
Hunter Drain	0.1	10.3	123	0.009	0.017	0.578	71	0.30	64.0	2140	3.23	0.097
Pintail Bay	0.80	36.1	24	<0.03	0.04	0.93	165	0.3	230.0	6730	2.42	<0.03
Lahontan Dam	<0.04	0.88	22	0.004	0.017	0.045	6	<0.01	2.5	33	0.234	0.047
Control Fresh	<0.04	0.22	73	0.006	0.019	0.028	25	<0.01	1.9	28	0.400	0.10
Control Salt												
5 ppth	<0.04	0.92	111	<0.003	0.028	0.036	181	<0.01	57.0	1470	1.59	0.14
12 ppth	<0.4	2.1	150	<0.03	<0.02	0.03	412	<0.1	130.0	3390	3.45	0.12

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Table 3. <u>Morone saxatilis</u>: Percent mortality of saltwater acclimated striped bass at 96 hours.

Percent Site Water	<u> Lead Lake</u>	Pintail Bay	Hunter Drain	TU Drain
12.5%	0	0	0	0
25.0%	0	20	0	20
50.0%	10	20	. 0	10
100.%	10	100	30	30
LC ₅₀ :	>100	50-100	>100	>100
	altwater cultu ilution/Lahonta	h): 20 10	·	

Table 4. <u>Pimephales promelas</u>: Percent mortality of freshwater cultured fathead minnows at 96 hours.

Percent Site Water	<u> Iead Iake</u>	Pintail Bay	Hunter Drain	TU Drain
12.5%	30	10	20	10
25.0%	20	20	0	30
50.0%	10	100	20	90
100.%	0	100	10	100
IC ₅₀ a:	>100	26	>100	28
	eshwater cultur lution/Iahontar			

aDoes not account for elevated mortality in dilution water.

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Table 5. <u>Hyalella azteca</u>: Percent mortality of saltwater (salt) and freshwater (fresh) cultured amphipods at 72 hours.

Percent:	<u>Iead Iake</u> <u>salt fresh</u>		<u>Pint</u>	ail Bay	<u>Hunter</u>	<u>Drain</u>	TU Drain	
Site Water			<u>salt</u>	salt fresh		salt fresh		salt fresh
12.5%	0	. 0	10	20	0	Ω	10	10
25.0%	10	0	20	0	Ö	0	0	10
50.0%	10	0	70	100	Ö	0	20	
100.%	10	10	100	100	_	_		0
100.0	10	10	100	100	0	0 -	20	10
1C ₅₀ :	>100	>100	31	35	>100 >	100	>100 >	>100
Controls: S	altwater reshwate				h): 5			

Table 6. <u>Daphnia magna</u>: Percent mortality of saltwater (salt) and freshwater (fresh) cultured daphnids at 48 hours.

Dilution/Iahontan (salt):
Dilution/Iahontan (fresh):

• •	<u> </u>	Pinta	ail Bay	Hunter	Hunter Drain		TU Drain	
Dilution: 12.5% 25.0% 50.0% 100.%	<u>salt fresh</u> 0 0 0 0 0 0 0 0 0 0	<u>salt</u> 0 0 100 100	fresh 0 0 100 100	<u>salt</u> 0 0 0 10	<u>fresh</u> 0 0 0 0	<u>salt</u> 0 0 90 100	fresh 0 0 100 100	
IC ₅₀ :	>100 >100	35	35	>100 >	100	38	35	
Controls:	Saltwater culture Freshwater culture Dilution/Lahontan Dilution/Lahontan	water (salt)	:	: 0 3 0 0				

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